Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 11, pp.22348-22353, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INTROGRESSION OF BLAST RESISTANCE GENES *Pi-54* AND *Pi1* INTO COLD TOLERANT VARIETY TELLAHAMSA, BY MARKER-ASSISTED SELECTION

[1]Jamal-Oddin, [*,1]Durga Rani, CH.V., [1]Swathi, G., [1]Anuradha, C.H., [1]Vanisri, S., [2]Sheshu Madhav, M., [2]Sundaram, R. M., [1]Arun Prem Kumar, N., [3]Krishnam Raju, S., [3]Bhuvaneswari, V., [4]Rajan, C. P. D., [5]Patibandla, A.K ., [6]Jagadeeswar, R., [7]Cheralu, C. and [3]Satyanarayana, P. V.

[1]Institute of Biotechnology, PJTSAU, Rajendranagar, Hyderabad, India
[2]Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad, India
[3]Andhra Pradesh Rice Research Institute (APRRI), West Godavari, India
[4]Agricultural Research Station, Nellore, India
[5]Regional Agricultural Research Station (RARS), Tirupati, India
[6]Agricultural Research Institute (ARI), Rice Section, Rajendranagar, Hyderabad, India
[7]College of Agriculture, Hyderabad, India

ARTICLE INFO

Article History:
Received 30th August, 2015
Received in revised form
27th September, 2015
Accepted 18th October, 2015
Published online 30th November, 2015

Key words:

Rice (Oryza sativa L.),
Blast resistance Pi-54 and Pi1, Marker assisted selection,
PCR, Uniform Blast Nursery.

ABSTRACT

Rice blast, caused by *Magnaporthe oryzae*, causes yield loss associated with injuries on leaves and necks. Broad spectrum introgression of blast resistance genes *Pi-54* and *Pi1* from the donor parent NLR145 (Swarnamukhi) into cold tolerant rice variety Tellahamsa (C10754) was carried out using marker assisted selection. The target genes were detected through the blast gene specific molecular marker *Pi-54* MAS for gene Pi54 and molecular marker RM224 which is closely linked to gene *Pi1* in F_1, BC_1F_1, BC_2F_1 and BC_2F_2 generations. In BC_2F_3, forty five progenies were analyzed through phenotypic assays with different blast pathogens at two blast hot spot regions west Godavari and Nellore. Four BC_2F_3 progenies possessing two blast resistance genes in homozygous condition (*Pi54Pi54 Pi1Pi1*) showed blast resistance along with Tellahamsa characteristics were advanced for multi location tests. This work demonstrates the successful application of molecular markers for targeted introgression of major blast genes *Pi-54* and *Pi1* in to a cold tolerant rice variety, Tellahamsa.

***Citation*:** Jamal-Oddin, Durga Rani, CH.V., Swathi, G., Anuradha, C.H., Vanisri, S., Sheshu Madhav, M. *et al.* 2015. "Introgression of blast resistance genes *Pi-54* and *Pi1* into cold tolerant variety tellahamsa, by marker-Assisted selection" *International Journal of Current Research*, 7, (11), 22348-22353.

INTRODUCTION

Rice is the most important food crop of the world. India is the largest rice growing country with second in production. It has a critical role in food security of Asia as 90% of the global production and more than 70% rice consumption, is by Asian countries. It accounts for 40% - 70% of the calories consumed by more than 40 billion Asian population (FAOSTAT 2012). Rice blast disease caused by *Magnaporthe oryzae* is one of the most destructive and wide spread disease (Jia *et al.,* 2000). The disease can strike all aerial parts of the plant, most infection occur on the leaves, causing diamond-shaped lesions with a gray or white center to appear on the panicles (Scardaci *et al.,* 2000).

Corresponding author: Durga Rani, CH.V.
Institute of Biotechnology, PJTSAU, Rajendranagar, Hyderabad, India.

Blast disease was first reported in Asia more than three centuries ago and prevalent throughout the continents where rice is cultivated. More than 85 countries are facing big yield loss due to this disease. Blast is a big money spinner, in many countries as farmers have been commonly using fungicides to protect the crop. However, clinical fungicides present hazards to human health and the environment. Blast controlling fungicides are expensive and involve in 6- 50% of the total plant protection cost. In India, among the biotic factors disease is the most important factor which results in crop losses of $5 billion every year (Asghar *et al.,* 2007). Due to blast, yield loss ranged from 1 to 50 %, meaning each year destroys abundant rice to feed more than 60 million people and economic loss over $70 billion of dollar (Scheuerman *et al.,* 2012). As the variations of pathogenicity of physiological races occurs more frequently, so some elite rice resistant varieties after cultivated for 4 or 5 years often gradually lost the resistance to rice blast.

As reported by CIAT, "New blast strains mutate rapidly, rendering resistant varieties susceptible within 2 or 3 years of release and sometimes, even before the breeding lines reach the farm (Fernando Correa- Victoria *et al.,* 1992). The result is a never ending race for breeders to keep ahead of the disease with new varieties. As these problems have become more widely recognized, the International Agriculture Research Institutions have responded by shifting their focus to breeding. Breeding efforts have also been quite limited in their success. Developing blast resistant varieties would be still the most cost- effective method to improve rice blast disease resistance in rice. Therefore, the use of resistant variety with multiple genes is thought to be one of the most economically and environmentally efficient way to avoid frequent break down of resistance. In adding to overlapping resistance, it could decrease the selection pressure on the pathogen and provide cross protection by minimizing the race evolution in the fungus.

To date, more than 85 blast resistance genes have been mapped, of which *Pib, Pita, Pid2, Pi9, Piz-t, Pi36* and *Pi37* has been isolated and cloned (Wu *et al.,* 2007; Qu *et al.,* 2006 and Lin *et al.,* 20007). *Pi-54*, is one of the major blast resistance gene and has been observed to show resistance against many isolates of the blast pathogen in India (Ramkumar *et al.,* 2011), which was considered as the widest spectrum resistance resource favored by the breeders in the cloned rice blast resistance genes. The resistance of rice varieties to rice blast was mostly controlled by a pair or several pairs of main effective dominant genes. The achievements have been made in applications of the blast resistance genes in rice blast resistant breeding program, such as the *Pid1, Pib*, *Pita* pyramided to G46B (Chen *et al.,* 20004), the *Pi-54*, *Pi2* introduced into B95-1(Ratna Madhavi *et al.,* 2013), the *Pi1, Pi2, Pi33 23B* introgressed to Jin 23B (Chen *et al.,* 20008), the *Piz5 and Pi-54* introduced into PRR78 (Vikas K. singh *et al.,* 2013), and a batch of new varieties possessing blast resistance were developed.

The present study has been carried out during 2009 to 2012. In this study an attempt was made to introgress *Pi-54* and *Pi1* genes in to Tellahamsa (C10754), a popular rice variety released from Professor Jayashankar Telangana State Agricultural University (PJTSAU), which is suitable for Rabi season because of its cold tolerance, high yielding ability and long slender grain quality. In our study Tellahamsa has been used as recurrent parent, while NLR145 containing *Pi-54 and Pi1* genes were used as donor parent. The main objective of the present research was to develop pre breeding lines containing blast resistance genes in the back ground of Tellahamsa.

MATERIALS AND METHODS

Materials used

Tellahamsa (C10754), a rice variety released from PJTSAU has become popular for Rabi season because of its cold tolerance, high yielding ability and long slender grain quality was used as recurrent parent and NLR145 containing *Pi-54 and Pi1* blast resistance genes was is used as donor parent.

Screening for leaf blast resistance

BC_2F_3 population was screened for blast resistance in Uniform Blast Nurseries at APRRI, Maruteru, West Godavari and Agricultural Research Institute, Nellore, Andhra Pradesh State, India.

DNA extraction and PCR analysis

The DNA was isolated following the modified CTAB (Cetyl Tri Methyl Ammonium Bromide) method (Murray *et al.,* 1980) The quality and quantity of DNA was estimated in 0.8% agarose gel using 500ug/ml lamda (λ) *Hind III* DNA (New England Biolabs) as reference standard. Fresh young rice leaves were taken into the 2ml eppendorf tube, adding liquid nitrogen to grind into powder and rapidly adding 600µl extract buffer incubating at 65**°c** water bath for 30~40 min, then adding 600µl mixture of chloroform and isoamylalcohol with 24 to1 and mixing at room temperature for standing 30 min.

After centrifuging at 10000 r/min for 15 min, the supernatant was transferred to another centrifuge tube, then an equal volume of chilled isopropanol was added and kept for 15min at -20°c for DNA precipitation, After centrifugation at 10000 r/min for 15 min, while precipitation was washed 2 times with 70% ethanol 100µl sterile water was used for dissolve to the naturally dried precipitate and placed in refrigerator at 4°c for ready to use. PCR was carried out to detect the presence of blast resistance gene *Pi-54* using *Pi54* MAS marker and *Pi1*gene using RM224 marker (Table 1) in the segregating population.

PCR amplification was performed in 10 µl of volume containing 10x PCR buffer (10 mM Tris–HCl (pH 8.0), 50 mM KCl), 1.5 mM $MgCl_2$, 2mM of dNTPs and 5 pmol of each forward and reverse primers, 5 units of *Taq* DNA polymerase (Genei, Bangalore, India), and 5ng of genomic DNA. Reactions were carried out in GenAmp PCR system 9700 (Applied Biosystem, USA), an initial denaturation at 94^{0}C for 5 minutes followed by cycle denaturation at 94^{0}C for 45 seconds; annealing step between 55^{0}C– 60^{0}C (according to the optimal temperature of the primers) for 45 seconds; extension at 72^{0}C for 60 seconds and with a final extension step at 72^{0}C for 10 min. The PCR mix was cycled 35 times. The PCR conditions were standardized by modifying annealing temperature of the

Table 1. Primers used for the identification of major blast resistance genes *Pi54* and *Pi1*

Resistance gene	Chr	Marker Name	Primers sequence used for gene identification	Expected size (bp)	Reference
Pi-54	11	Pi54 MAS	CAATCTCCAAAGTTTTCAGG-F GCTTCAATCACTGCTAGACC-R	200	Ramkumar *et al.,* 2011
Pi1	11	RM224	ATCGATCGATCTTCACGAGG-F TGCTATAAAAGGCATTCGGG-R	130	Hittalman *et al.,* 2000)

The PCR amplified samples were then mixed with bromophenol blue and run on 3% Agarose gel along with the 1000ug/ml 50-bp DNA ladder (New England Biolabs) for two hours in 0.5x Tris-Acetic acid EDTA (TAE) buffer. The DNA

fragments were visualized under UV-Transilluminator and documented using Bio-Rad Molecular Imager Gel Doc XR System (Universal Hood II) and image was saved for further analysis.

RESULTS AND DISCUSSION

Transferring Blast resistance genes by MAS

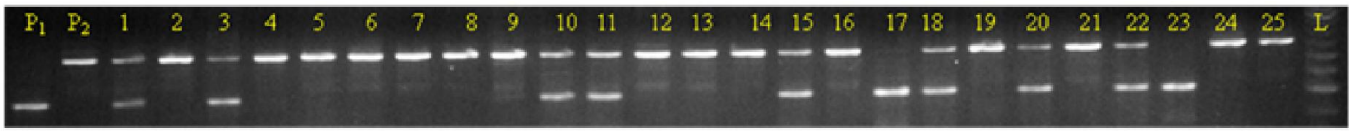
Marker assisted selection (MAS) has been successfully applied for improving resistance against biotic stresses like blast, bacterial blight and BPH in rice (Joseph *et al.,* 2004, Sundaram *et al.,* 2008, Hari *et al.,* 2011, Hari *et al.,* 2013, Chen *et al.,* 20004 and Khanna *et al.,* 2015) as MAS saves time and offers a very simple efficient and accurate method to improve the blast resistance of elite genotype (Singh *et al.,* 2012). In our present study, the F_1 plants with heterozygous alleles of two blast resistance genes (*Pi-54* and *Pi1*) were obtained from the cross of susceptible Tellahamsa (C10754) as recurrent parent and NLR145 as donor parent. The heterozygocity was confirmed by using a gene specific marker *Pi54* MAS for *Pi54* gene and a gene linked marker, RM224 for *Pi1* gene were employed to perform marker assisted selection in F_1 and BC_1F_1 generations.

The marker *Pi54* MAS amplified a fragment of 200bp in NLR145 (Resistance specific band) and 350bp fragment in Tellahamsa (Susceptibility specific band), while RM224 amplified a fragment of 130 bp in NLR145 (R) and 150 bp fragment in Tellahamsa (S).

Genetic analysis of 202 BC_1F_1 plants for *Pi54* gene revealed 64 plants as heterozygous, while 86 plants showed heterozygocity for *Pi1* gene. Thus a total of 31 BC_1F_1 plants showed heterozygocity for both genes on using both markers. The advanced back cross plants of BC_2F_1 were obtained from the cross made between selected BC_1F_1 and Tellahamsa. Foreground analysis showed that 154 plants as heterozygous of 352 BC_2F_1 plants studied for *Pi-54* gene, while 188 plants out of 352 plants were observed as heterozygous for *Pi1* gene (Fig. 1). A total of 38 BC_2F_1 plants showed heterozygocity for both the genes. Selection was carried out in 38 genes positive BC_2F_1 plants based on phenotypic and other grain characters and one BC_2F_1 plant was allowed for selfing and advanced as BC_2F_2 population for future study.

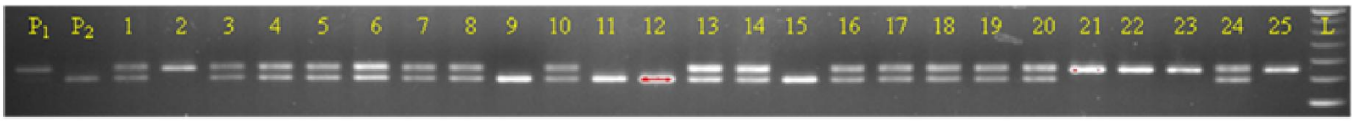
Genetic analysis of blast resistance genes (*Pi54* + *Pi1*) in BC_2F_2 population

Genotyping was carried out in four hundred and eleven BC_2F_2 plants. DNA was extracted from these 411 plants and subjected to genotypic analysis with the *Pi54* gene specific marker *Pi54* MAS. Out of 411 BC_2F_2 plants, 103 plants were homozygous resistant (RR), 196 plants were heterozygous resistant (Rr), and 112 plants were homozygous susceptible (rr) (Fig. 3). Later these 411 plants were subjected to genotypic analysis with RM224 marker specific for *Pi1* gene. Out of 411 BC_2F_2 plants, 105plants were homozygous resistant (RR), 198 plants were heterozygous resistant (Rr), and 108 plants were homozygous susceptible (rr) (Fig. 4). The population segregated in a true Mendelian way (i.e. 1:2:1).



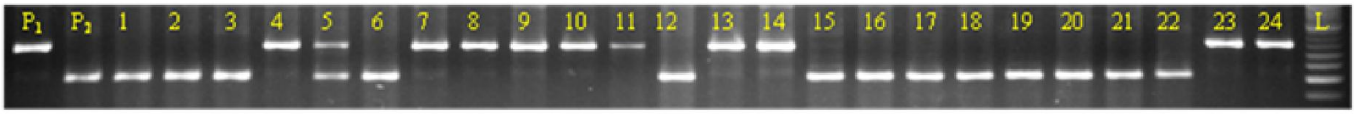
P1= NLR145 P2= Tellahamsa L= 50bp ladder and herozygotes in lane-1, 3, 10, 11, 15, 18, 20 and 22

Figure 1. Confirmation of TH X NLR145 BC_2F_1 plants by *Pi54* MAS primer



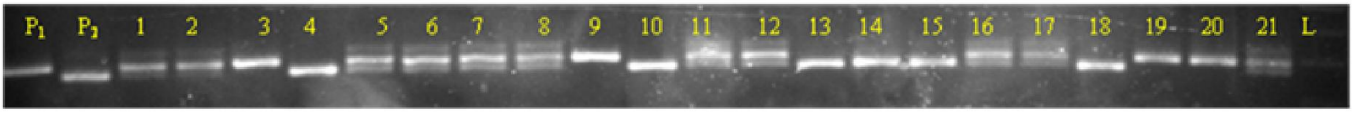
P1= Tellahamsa, P2= NLR145, L= 50bp ladder and herozygotes in lane-2: 1, 3, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 18, 19, 20 and 24
Identified common heterozygotes in lane-1 and lane-2 : 1, 3, 10, 18 and 20

Figure 2. Confirmation of TH X NLR145 BC_2F_1 plants by RM 224 primer



P1= Tellahamsa, P2= NLR145, L= 50bp ladder and homozygous in lane-3 : 1, 2, 3, 6, 12, 15, 16, 17, 18, 19, 20, 21 and 22

Figure 3. Confirmation of TH X NLR145 BC_2F_2 plants by *Pi54* MAS primer



P1= Tellahamsa, P2= NLR145, L= 50bp ladder and homozygous in lane-4 : 4, 10, 13, 14, 15 and 18
Identified common homozygous in lane-3 and lane-4 : 15 and 18

Figure 4. Confirmation of TH X NLR145 BC_2F_2 plants by RM 224 primer

Similar results are obtained by (Fatah *et al.,* 2015, Ratna *et al.,* 2012 and Immanuel *et al.,* 2011). When phenotypic data was correlated with genetic analysis data, a total of two recombinants were observed among homozygous resistant plants. Results revealed that 45 plants were positive for both the genes (*Pi54 + Pi1*) and these plants (homozygous for both *Pi54* and *Pi1*) were selfed and advanced to BC_2F_3 generation.

Screening of BC_2F_3 progenies for blast resistance

Artificial screening for rice blast was carried out in 45 BC_2F_3 progenies during Rabi, 2012 at two hot spot locations of Andhra Pradesh (APRRI, RARS, Maruteru, West Godavari district and ARS, Akuthota, Nellore district). A local isolate of *Magnaporthe oryzae* from APRRI, RARS, Maruteru, West Godavari and another local isolate of *Magnaporthe oryzae* from RARS, Akuthota, Nellore district, Andhra Pradesh , India, was used to screen the donor and recurrent parent along with BC_2F_3 progenies under in vivo conditions following uniform blast nursery (UBN) method.

The young seedlings at four leaf stage were inoculated with fungal conidial suspension at a concentration of $1x10^5$ conidial/ml was sprayed and UBN beds are covered with polythen sheets during night time for high relative humidity was maintained for disease development (Fig. 5). The disease reaction was recorded 15 days after inoculation on each plant following on the basis of the IRRI-SES 0-9 scale (IRRI, 1996). Fifteen BC_2F_3 progenies showed resistance reaction (0, 1-3 and 4-5 disease score) to blast disease at two locations.

Among them four progenies showing close resemblance to Tellahamsa based on plant height (95cm), 50% flowering duration (90days) and grain type (Long slender) were advanced to multi locations trials. The results revealed that of the phenotyping screening against blast disease reaction of the BC_2F_3 progenies carried the *Pi-54* and *Pi1* genes with the characteristics of a back ground of the recurrent parent Tellahamsa conferred highly resistance against two different blast pathogens at two blast hot spot regions west Godavari and Nellore.

Table 2. BC_2F_3 progenies against blast resistance in different hot spot locations of Andhra Pradesh Provinces West Godavari district (Maruteru) and Nellore District (Akuthota)

Cross	Total No. of Genotype/ progenies	Frequency distribute of disease score at West Godavari					Observed frequency phenotypic data	
		0	1-3	4-5	6-7	8-9	R	S
TH	1	0	0	0	0	1	0	1
NLR145	1	0	1	0	0	0	1	0
TH/NLR145 BC_2F_3	45	3	12	11	18	2	26	19

Cross	Total No. of Genotype/ progenies	Frequency distribute of disease score at Nellore					Observed frequency phenotypic data	
		0	1-3	4-5	6-7	8-9	R	S
TH	1	0	0	0	0	1	0	1
NLR145	1	0	1	0	0	0	1	0
TH/NLR145 BC_2F_3	45	2	9	13	11	10	24	21

For phenotypic data, disease score up to 5 were taken as resistant and plants with score greater than 5 were taken as susceptible. R=Resistant, S= Susceptible

Fig. 5. UBN beds covered with polythen sheets during night time for disease development

Acknowledgement

We heartily thankful to Department of Biotechnology (DBT), India, for providing research grants and PJTSAU for providing facilities.

REFERENCES

Asghar, A.H., Rashid, M., Ashraf, M.H., Khan and Chaudhry, A.Z. 2007. Improvement of basmati rice, against fungal infection through gene transfer technology, *Pak.J. Bot.,* 39(4): 1277-83.

Chen, H.Q., Chen Z.X., Ni S., Zuo S.M., Pan X.B., and Zhu X.D. 2008. Pyramiding Three Genes with Resistance to Blast by Marker-Assisted Selection to Improve Rice Blast Resistance of Jin 23B, application, Zhongguo Shuidao Kexue. *Chinese Journal of Rice Science*.

Chen, X.W., Li S.G., Ma, Y.Q., Li, H.Y., Zhou, K.D., and Zhu, L.H. 2004. Marker-assisted selection and pyramiding for three blast resistance genes, *Pi-d*(*t*)*1*, *Pi-b*, *Pi-ta2*, in rice, Shengwu Gongcheng Xuebao. *Chinese Journal of Biotechnology,* 20(5): 708-714.

Fatah, A., Tanweer , Mohd, Y. Rafii, K. Sijam , Harun A. Rahim, F. Ahmed, Sadegh Ashkani and Mohammad, A. Latif. 2015. Introgression of blast resistance genes (putative Pi-b and Pi-kh) into elite rice cultivar MR219 through Marker-Assisted Selection. Front. *Plant Sci*., 6:1002. doi:10.3389/fpls.2015.01002.

Fernando Correa Victoria *et al.,* 1992. Know Your Enemy: A novel strategy to develop durable resistance to rice blast fungus through understanding the genetic structure of the pathogen population, CIAT.

Hari, Y., Srinivasa Rao, K., Viraktamath B.C., Hariprasad, A.S., Laha, G.S., Ahmed, M., Nataraj Kumar, P., Sujatha, K., Srinivasprasad, M.S., Rani, N.S., Balachandran, S.M., Kemparaju, S., Mohan, K.M., Sama, V.S.A.K., Shaik, H., Balachiranjeevi, C.H., Pranathi, K., Reddy, G.A., Madhav, M.S. and Sundaram, R.M. 2013. Marker-assisted introgression of bacterial blight and blast resistance into IR 58025B, an elite maintainer line of rice. *J. Plant Breed.*, 132:586–594.

Hari, Y., Srinivasa Rao, K., Viraktamath, B.C., Hariprasad, A.S., Laha, G.S., Ilyas Ahmed, M., Nataraj Kumar, P., Ramesha, M.S., Neeraja, C.N., Balachandran, S.M., Shobha Rani, N., Balaji Suresh, P., Sujatha, K., Pandey, M., Ashok Reddy, G., Madhav, M.S. and Sundaram, R.M. 2011. Marker-assisted improvement of a stable restorer line, KMR-3 Rand its derived hybrid. 130: 608- 616.

Immanuel Selvaraj, C., Pothiraj Nagarajan, Thiyagarajan, K., Bharathi, M. and Rabindran, R. 2011. Identification of microsatellite (SSR) and RAPD markers linked to rice blast disease resistance gene in rice (*Oryza sativa* L.), *African Journal of Biotechnology*, 10(17): 3301- 3321.

Jia, Y., Me Adams, S.A., Bryan, G.T., Hershay, H.P. and Valent, B. 2000. Direct interaction of resistance genes products confers rice blast resistance. *Embo. J.,* 19:4004 – 4014.

Joseph, M., Gopala Krishnan, S., Sharma, R.K., 2004. Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Mol Breed.,* 13:377–387.

Khanna, A., Sharma, V., Ellur , R.K., Shikari, A.B., Gopala Krishnan, S., Singh, U.D., Prakash, G., Sharma, T.R., Rathour, R., Variar, M., Prashanthi, S.K., Nagarajan, M., Vinod, K.K., Bhowmick, P.K., Singh, N.K., Prabhu, K.V., Singh, B.D. and Singh, A. K. 2015. Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. *Theor Appl Genet*., doi:10.1007/s00122-015-2502-4.

Lin, F., Chen, S., Que, Z.Q., Wang, L., Liu, X.Q. and Pan, Q.H. 2007. The blast resistance gene *Pi37* encodes a nucleotide binding site-leucine-rich repeat protein and a member of a resistance gene cluster on rice chromosome 1. *Genetics*., 177(3): 1871-1880.

Murray, M.G. and Thompson, W.F. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res. 1980. 8: 4321– 4325.

Qu, S.H., Liu, G.F., Zhou, B., Bellizzi, M., Zeng, L., Dai l., Han, B., and Wang G.L. 2006. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multi-gene family in rice, *Genetics,* 172(3): 1901-1914.

Ramkumar, G., Srinivasarao, K., MadanMohan, K., Sudarshan, I., Sivaranjani, A.K.P., Gopalakrishna, K., Neeraja, C.N., Balachandran, S.M., Sundaram, R.M., Prasad, M.S., Shobha Rani, N., RamaPrasad A.M., Viraktamath, B.C. and Madhav, M.S. 2011. Development and validation of functional marker getting an InDel in the major rice blast disease resistance gene Pi54 (Pikh). *Mol. Breeding*., 27(1): 129- 135.

Ratna Madhavi, K., Srinivas Prasad, M., Sheshu Madhav, M., Laha, G. S., K Madhan Mohan, Sundaram, R.M., Jahnavi, B., Vijitha, S., Rao, P. R. and Viraktamath, B.C. 2012. Introgression of blast resistance gene Pi-kh into elite Indica rice variety improved samba mahsuri. *Indian Journal of Plant Protection,* 40(1): 52-56.

Scardaci, S.C. *et al.,* 2000. Rice Blast: A New Disease in California. Agronomy Fact Sheet Series 1997-2, Department of Agronomy and Range Science, University of California, Davis, 18 May 2000.

Scheuerman, K.K., Raimondi, J.V., Marschalek, R., Andrade, A. and Wickert, E. 2012. *Magnaporthe oryzae* genetic diversity and its outcomes on the search for durable resistance. *Mol Basis Plant Genetic Divers*., 31–356.

Singh, V.K., Singh, A., Singh, S.P., Ellur, R.K., Choudhary, V., Singh, D., Gopala Krishnan, S., Nagarajan, M., Vinod, K.K., Singh, U.D., Prashanthi, S.K., Agrawal, P.K., Bhatt, J.C., Mohapatra, T., Prabhu, K.V., Sarkel, S., Rathore, R. and Singh, A.K. 2012. Incorporation of blast resistance into ‘‘PRR78’’, an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crops Res*., 12:8–16.

Standard evaluation system for rice. International Rice Testing Program. International Rice Research Institute, Philippines. 1996.

Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, G.A., Rani, N.S., Sarma, N.P. and Sonti, R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*., 160:411–422.

Vikas K. Singh, Atul Singh, S. P., Singh, Ranjith, K., Ellur, Devinder Singh, S., Gopala Krishnan, P.K., Bhowmick,

M., Nagarajan ,K. K., Vinod, U. D., Singh, Mohapatra, T., Prabhu, K. V. and Singh, A. K. 2013. Marker-assisted simultaneous but stepwise backcross breeding for pyramiding blast resistance genes Piz5 and Pi54 into an elite Basmati rice restorer line 'PRR78' DOI: 10.1111/pbr.12077.

Wu, J., Liu, X.L., Dai, L.Y. and Wang G.L. 2007. Advances on the identification and characterization of broad-spectrum blast resistance genes in rice, Shengming Kexue *Chinese Bulletin of Life Sciences*, 19(2): 233-23.

****